A COMPARATIVE STUDY OF THE LIFE HISTORY OF THE ARKANSAS, DELICIOUS, AND GRIMES GOLDEN APPLES WITH RESPECT TO THE VARIATIONS IN FRUITFULNESS SHOWN BY THESE VARIETIES

BY

LEON FREDRIC HOUGH

B.S., Michigan State College, 1938
M.S., Cornell University, 1939

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN HORTICULTURE
IN THE GRADUATE SCHOOL OF THE
UNIVERSITY OF ILLINOIS, 1947

URBANA, ILLINOIS

1947

A COMPARATIVE STUDY OF THE LIFE HISTORY OF THE ARKANSAS, DELICIOUS, AND GRIMES GOLDEN APPLES WITH RESPECT TO THE VARIATIONS IN FRUITFULNESS SHOWN BY THESE VARIETIES

BY

LEON FREDRIC HOUGH

B.S., Michigan State College, 1938
M.S., Cornell University, 1939

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN HORTICULTURE
IN THE GRADUATE SCHOOL OF THE
UNIVERSITY OF ILLINOIS, 1947

URBANA, ILLINOIS

1947

ACKNOWLEDGMENTS

The investigations herein reported were planned and conducted under the supervision of Dr. M. J. Dorsey, who made the necessary facilities of the Department of Horticulture at the University of Illinois available for this work. The writer is deeply grateful to Dr. Dorsey for his continuing interest and encouragement during the course of these investigations.

Credit is due the personnel of the Division of Plant Breeding for help during certain phases of the work, particularly to those who assisted with the cross-pollinations; and to Miss Katherine M. Glasgow and Mr. James B. Mowry for assistance with the technical routine. Credit is also due my wife, Mary Y. Hough, for critical reading of the manuscript.

R. UNIV.
BIBLIOTHEEK
LEIDEN

A COMPARATIVE STUDY OF THE LIFE HISTORY OF THE ARKANSAS, DELICIOUS, AND GRIMES GOLDEN APPLES WITH RESPECT TO THE VARIATIONS IN FRUITFULNESS SHOWN BY THESE VARIETIES

The finding of a large number of triploids among the commercial varieties of apples, together with the cytological irregularities that have been associated with these varieties in common with triploid plants generally, has too frequently resulted in the assumption that wherever imperfect gametophytes were a factor in reducing fruitfulness in a variety, triploidy was the sole cause.

From a consideration of the influence genetic characters have been shown to exert on different stages of development in living organisms, it may be postulated *a priori*: that there are genetic factors other than triploidy *per se* influencing sporogenesis which may reduce the amount of functional pollen and embryo sacs in varieties of apples. Similarly, the factor or factors affecting pollen development may be distinct from those affecting the development of the embryo sac.

This investigation was undertaken to determine whether such differences in the genetic controls of the several stages in the life cycle of apples could be demonstrated.

Material and Methods

The Arkansas, Delicious and Grimes Golden apple varieties were selected for study on the basis of the known differences in their cytological and pomological behavior. Arkansas is

a triploid variety which produces pollen of low viability and which is unreliable in fruit production. This "capriciousness" in fruit setting has been attributed to the irregularities during meiosis whic hare a consequence of its triploid constitution. Delicious is a diploid variety which is recognized as an excellent source of pollen but which often suffers an excessively heavy first drop as a result of imperfect and unfertilized ovules. Grimes Golden is a diploid variety which produces normal pollen and which is pomologically classified among the heavy setting varieties.

The trees of the three varieties which were used for this study were mature trees growing in sod or sod with cultivation between the tree rows. The trees received a minimum of pruning consistent with commercial spraying and orchard management practices. No nitrogenous fertilizer was applied during or immediately prior to this investigation. However, the trees selected each season were of moderate vigor and had made satisfactory growth the preceding season. In most cases the trees were blosoming fully enough to give a heavy commercial crop.

At one time or another during the springs of 1942, 1943, 1944, and 1945 injury from frost or lack of pollination because of cold rainy weather interfered with a comparative study of the development of the sexual elements. Nevertheless normal collections were obtained for each of the various stages up to bloom during some one of the four seasons. Normal collections from anthesis up to fertilization were obtained from branches which were forced in the greenhouse during the spring of 1946.

Collections of at least ten lateral flowers were taken at one or two day intervals from the beginning of microsporogenesis up until anthesis and at daily intervals after pollination. Because of inclement weather the complete series of collections were not obtained in any one season.

Iron-aceto-carmine or iron-propiono-carmine smears were

made to determine the proper time to collect material for the early stages of microsporogenesis. In 1942 all buds were killed in Carony's fluid (3 parts absolute alcohol to 1 part glacial acetic acid) for about 15 minutes. They were then placed in Randolf's CRAF fixative and evacuated for 15 to 20 minutes, after which they were stored in the CRAF solution until it was convenient to dehydrate and imbed them. In later seasons propionic acid was substituted for the acetic acid in each fluid with no noticeable effect on the quality of fixation.

After washing out the fixing solution, all buds were carefully scraped free of epidermal hairs both on the outside and on the inside of the flower around the base of the style. Whole buds were used as far as practicable in order to be able to compare the development of the anthers and ovules within a given flower. The material was dehydrated with tertiary butyl alcohol and embeded in a rubber-ceresin-paraffin mixture. Microsporogenesis stages were sectioned at 8 to 10 microns, megasporogenesis stages and young embryo sacs at 15 microns and later stages were sectioned at 22 microns. All the material was stained with dilute (0.05 per cent) aqueous crystal violet and picric acid in absolute alcohol.

Observations

Anthers. Meiotic divisions were first observed in the anthers of flowers from flower clusters at the late delayed dormant or green tip stage of development and these stages could be found for several days thereafter. The primordia of the ovules could first be observed in the carpels at this time. The early stages of the first meiotic prophase were of much longer duration than the subsequent meiotic stages. In all three varieties the chromosome threads formed a dense synaptic mass at one side of the nucleus during mid-prophase of the first meiotic division. Individual chromosomes could not be distinguished at this stage, but typical

early diakinesis configurations with from one to several chiasmata were evident following synapsis.

The behavior of the chromosomes of Delicious and Grimes Golden in the subsequent stages of meiosis was typical of that found in diploid varieties. In the triploid, Arkansas, the behavior of the chromosomes was less regular. The number of bivalent and trivalent groups was not constant, and the separation of the chromosomes at early anaphase was very irregular although no evidences of structural hybridity were observed. Nevertheless the late anaphase and telophase stages indicated that in spite of the uneven beginning of division, considerable regularity prevailed so that most of the chromosomes were finally included in the daughter nuclei. Laggards were also observed during anaphase of the second meiotic division but here too only a few chromosomes were left in the cytoplasm after the telophase nuclei were formed.

The mitotic division of the microspore nucleus occurred at the cluster bud or pre-pink stage of development of the flowers, and the megaspore mother cells in the ovules of the same flowers were in prophase stages of the first meiotic division at this time. Some abortion occurred during the development of the pollen of Arkansas, and to a lesser extent of Delicious and Grimes Golden as well. The triploid variety was remarkable in exhibiting considerable regularity during the development of the pollen and in producing a large proportion of normal appearing pollen grains, but in spite of this the germination of the Arkansas pollen was only one fifth as good as that of the diploid varieties. At the time of pollen germination two vermiform nuclei were present in the pollen grain.

Ovules. The behavior of the chromosomes during megasporogenesis very closely paralled the behavior of the chromosomes during microsporogenesis. It was regular in Delicious and Grimes Golden and some lagging was observed in Arkansas, but this did not prevent the regular production

of a high proportion of tetrads in the triploid variety. The development of the embryo sac from the chalazal megaspore followed the pattern of the normal development typical of angiosperms. In Grimes Golden this pattern was observed regularly and the same developmental sequence was observed frequently in the ovules of Arkansas and Delicious, but the many exceptions to this behavior in these latter varieties indicated that in these two varieties this developmental sequence was not rigidly controlled.

In the development of the ovules of Delicious, the most frequent abnormality was either a tardy initiation of the megaspore mother cell or a slower rate of development of the megaspores and the embryo sac. Such retarded embryo sacs would seldom be expected to develop into fully differentiated eight-nucleate embryo sacs in time for fertilization, especially if their development continued to be at a slower than normal rate. Other apparently normal embryo sacs broke down soon after the flower opened even though the flower had been pollinated with compatible pollen. Less frequently the megaspores or young embryo sacs collapsed. In every case where the megaspores or embryo sac failed to develop fully—and in some ovules where the embryo sac appeared to be normal—secondary megaspores or embryo sacs were differentiated in the nucellar axis obove the primary megaspores or embryo sac. These secondary embryo sacs, like the retarded primary embryo sacs, would rarely be expected to develop into a mature embryo sac in time for fertilization. The frequent evidence of nuclei in early spireme, synapsis or diakinesis stages in the cells in the nucellar axis is evidence that these secondary megaspores were produced through meiotic division.

In the development of the ovules of Arkansas various degrees of lack of differentiation of the eight nuclei of the embryo sac were observed. Occasionally megaspores or embryo sacs broke down. Just as in Delicious, wherever megasporogenesis or megagametogenesis did not proceed

normally, secondary megaspore mother cells were differentiated among the cells of the nucellar axis, and these often continued to develop normally even though they would not form functional secondary embryo sacs in time for fertilization.

Twin embryo sacs were observed in two instances in Grimes Golden, but in both of these ovules it was evident that the embryo sacs had developed simultaneously.

During the growth of the ovule up until the time of fertilization, a few cells in the nucellar axis above the megaspores or developing embryo sac remained potentially sporogenous. They were conspicuous because of their larger size, denser cytoplasm and larger, darker staining nuclei. These were the cells which functioned as secondary megaspore mother cells when this abnormality occurred within the ovule.

Discussion

From the observations reported, certain generalizations may be made with respect to the significance of the similarities and differences which occurred during the life cycles of the apple varieties studied.

Chromosome morphology. During the prophase stages of the first meiotic division the structure and significant cyto-genetic behavior of apple chromosomes was found to be comparable to that of other plants such as corn which have been investigated more fully, but the close synaptic association of all the chromosomes of the apple during mid-prophase precludes a detailed analysis of their morphology.

Activity in the nucellar axis. It is suggested that the transfer of nutrients from the chalazal extremity of the vascular bundle to the megaspores or developing embryo sac in the young ovule results in the establishment of a

nutritional gradient along the nucellar axis, and that the advantageous location of the cells in the nucellar axis with respect to this gradient would in a large measure account for their ability to remain potentially sporogenous. This same argument would account for the fact that—except for cases of extreme genetic disparity between the megaspores resulting from segregation in the course of the meiotic divisions—the chalazal megaspore, which is more advantageously located with respect to the nutrient supply, invariably develops into an embryo sac. Following fertilization the rapid growth of the endosperm and elongation of the embryo sac allows the endosperm to replace the cells of the nucellar axis as a channel for the transfer of food materials from the chalazal tip of the vascular bundle to the embryo in the micropylar portion of the ovule.

Diploid development. Pomologists have recognized the fact that the physiological condition of the flower is important in determining the course of development of the ovules, and it has been shown that extremes of vigor will have a differential effect on the development of the male and female sexual elements of flowers. However the abnormalities observed during megasporogenesis and the development of the female gametophyte in this study were present in flowers taken over a period of five seasons from several trees all in moderately good vigor. No obvious nutritional gradients were operating which would allow regular sporogenesis and development of the gametophyte in Delicious anthers and still bring about the considerable irregularities observed during sporogenesis and development of the gametophyte in Delicious ovules. This contrast is emphasized by the fact that regular development was observed in both the anthers and ovules of Grimes Golden which were produced under comparable nutritional conditions.

Therefore since it is not likely that physiological conditions were principally responsible for the abnormalities observed, and since genetic factors have frequently been

shown to cause abnormal sporogenesis or degeneration during the development of the gametophyte in fruits and other plants, it is suggested that the abnormalities which were observed during the development of the ovule in Delicious are due to the effect of one or more genetic factors. It is further suggested that the genetic factor or factors result in a modification of the normal genetic controls of megasporogenesis and the development of the embryo sac, and are presumably effective through the maternal tissue.

Triploid development. It has already been pointed out that some irregularities, principally lagging chromosomes, occur during meiosis in Arkansas. These irregularities are typical of triploids and result in the production of a high proportion of aneuploid gametes. Presumably certain of these aneuploid gametes contain such unbalanced genetic combinations that the gametophytes cannot develop normally. This would account for the higher percentage of aborted pollen in Arkansas as compared to the diploid varieties, and it would also account for the occasional breakdown of megaspores. Aneuploid gametophytes might also be produced which could develop but could not function normally as a result of a slightly less violent genetic unbalance of the aneuploid number of chromosomes in their nuclei. Such a condition would account for the high proportion of morphologically normal but non-viable pollen, and for the lack of differentiation in many of the embryo sacs.

Nevertheless many of the irregularities that have frequently been reported during meiosis in other triploid varieties typically do not occur in Arkansas. Furthermore the abnormalities observed in the development of the embryo sac of this triploid variety rather closely parallel the abnormalities observed in the development of the embryo sac in Delicious, a diploid variety. Consequently, while it is recognized that much of the irregularity which occurs during sporogenesis and the development of the gametophytes in Arkansas is a direct consequence of its triploid chromosomal

constitution and of the aneuploid chromosomal constitution of the resulting spores and gametes, it is suggested that the degree to which these irregularities are expressed may be subject to modification by genetic controls which are probably operative through the maternal tissue.

It is further suggested that the arguments presented support the general hypothesis that there are genetic factors other than triploidy *per se* which may affect sporogenesis and influence the amount of functional pollen and embryo sacs produced by varieties of apples.

In conclusion, it is recognized that further critical study of the life history of these varieties would be desirable, particularly in indicating the manner and extent to which cultural and environmental conditions can modify the expression of the genetic controls concerned with these processes.

VITA

Leon Fredric Hough was born at Romeo, Michigan, on July 14, 1915. He was graduated from Romeo High School in 1933. He received the degree of Bachelor of Science with honor from Michigan State College in 1938.

He was appointed a part-time teaching assistant in the Department of Pomology at Cornell University in 1938 and received the degree of Master of Science from Cornell University in 1939. He continued his teaching assistantship and graduate study toward the Ph. D. degree until 1941.

During the summers of 1939 and 1940 he assisted in the fruit breeding work of the Division of Pomology of the New York State Agricultural Experiment Station at Geneva, New York, as an agent of the United States Department of Agriculture.

In 1941 he came to the University of Illinois as a full-time assistant in the Department of Horticulture, and was enrolled in the Graduate School.

He was elected to Alpha Zeta in 1936, Seminarium Botanicum in 1937, Phi Sigma in 1942, Pi Mu Epsilon in 1944, Gamma Sigma Delta, the Honor Society of Agriculture in 1946, Phi Kappa Phi and the Society of the Sigma Xi in 1947.

PUBLICATIONS

Hough, L. F. A study of the value of certain apple varieties as pollinizers for McIntosh and Rhode Island Greening. Master's Thesis, Cornell University 1939, 46 pp.

Hough, L. F. The pollen value of 134 apple varieties as determined by germination tests and field trials. Proc. Amer. Soc. Hort. Sci.**37**: 133-136. 1940.

Hildebrand, E. M. and L. F. Hough. Pollenicides as supplements for bactericides in blossom blight control. Proc. Amer. Soc. Hort. Sci. **40**: 91-94. 1942.

Dorsey, M. J. and L. F. Hough. Relation between seedling vigor and tree vigor in apple hybrids. Proc. Amer. Soc. Hort. Sci **43**: 106-114. 1943.

Hough, L. F. and E. L. Welker. Combining genetically different samples for correlation and analysis. Proc. Amer. Soc. Hort. Sci **43**: 155-159 1943.

Hough, L. F. A survey of the scab resistance of the foliage on seedlings in selected apple progenies. Proc. Amer. Soc. Hort. Sci. **44**: 260-272. 1944.

Hough, L. F. The new pear breeding project. Trans. Ill. State Hort. Soc. **78**: 106-110. 1945.